3 MATERIALS AND METHODS

3.1 Overview

This chapter will discuss more details about the procedures involved in this research which including the material used, methods conducted during the experiment and also analysis that have been done after the experiment.

3.2 Introduction

Materials used in this research consisted of bacteria, chemicals and metals. Meanwhile, the methods involved were preservation of stock culture, media preparation, culture preparation, and also cell – surface adhesion experiment. The analysis parts were divided into a few category which were, cell concentration measurement (Optical Density, OD), Colony Forming Unit (CFU), staining process (Fluorescence dye-SYTO9), quantifying attached cell using fluorescence microscope, fixing and preparation of samples for Scanning Electron Microscopy (SEM) analysis and lastly counting and morphological observation of adhered microorganism using SEM. The overall process involved in this experiment has been summarized in Figure 3-1.

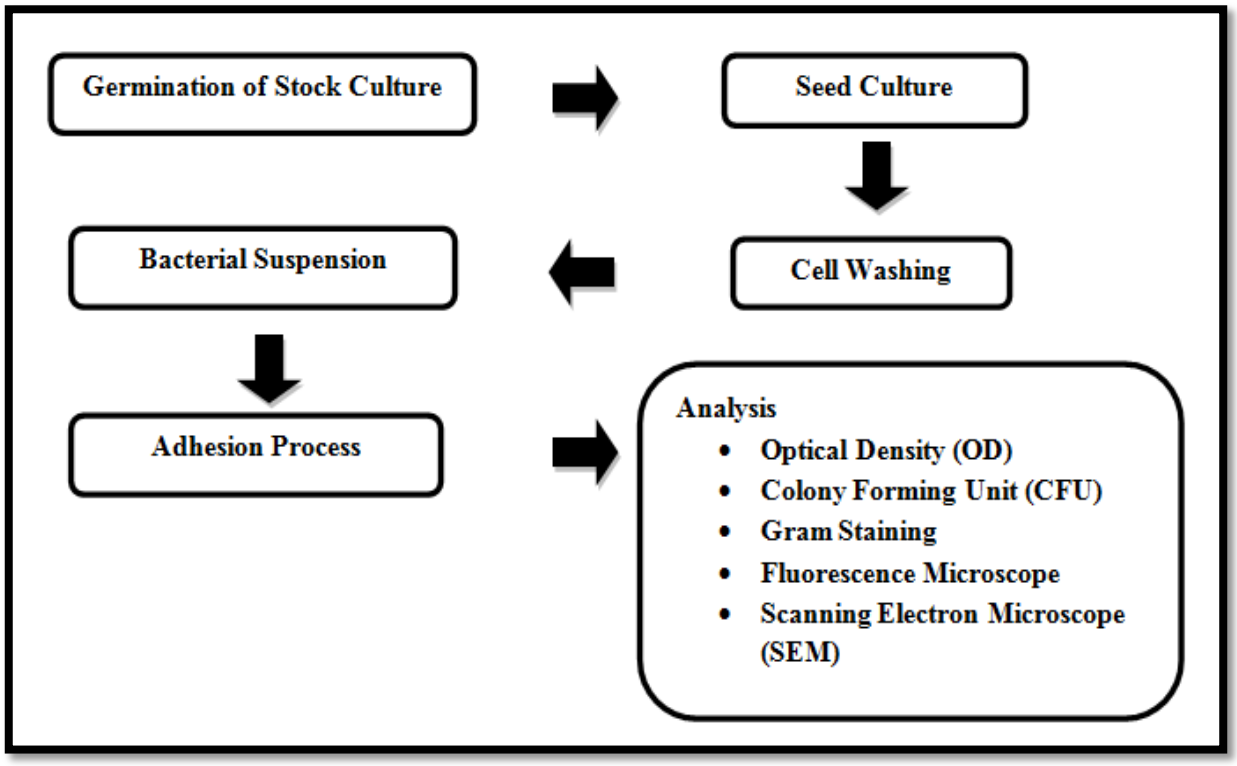

Figure 3-1: Flow chart for adhesion process

3.3 Materials

3.3.1 Bacteria

Three types of bacterial were used in this study which were *Escherichia coli, Bacillus subtilis* and *Staphylococcus aureus.* All the bacterial were obtained from the Centre Laboratory of Universiti Malaysia Pahang and kept in the FKKSA laboratory chiller at 4-6 °C before handling the experiment.

3.3.2 Chemicals

Glucose, bactopeptone, yeast extract, agar powder, sulphuric acid, peptone from caseine, ethanol, NaOH, K_2HPO_4, KH_2PO_4, KCl, $MgSO_4$, NaCl and glutaraldehyde were obtained from FKKSA Laboratory, UMP. All the chemicals were purchased from Sigma-Aldrich, Malaysia and were of biological grade.

3.3.3 Metals

There were three types of metals used in this study which were Stainless Steel (N690), Stainless Steel (AV220SC) and Titanium. Each of the metal was consisted eight different types of roughness. All the metals were put in the bacterial solution and undergo adhesion test for 4 hours. Below is the image (Figure 3-2) of metals used in this study. The symbols P1, P2, P3, P4, E1, E2, E3 and E4 denoted the types of metals surface roughness used in this study and at each of surface roughness contained three different types of metals as shown in the Figure 3-2.

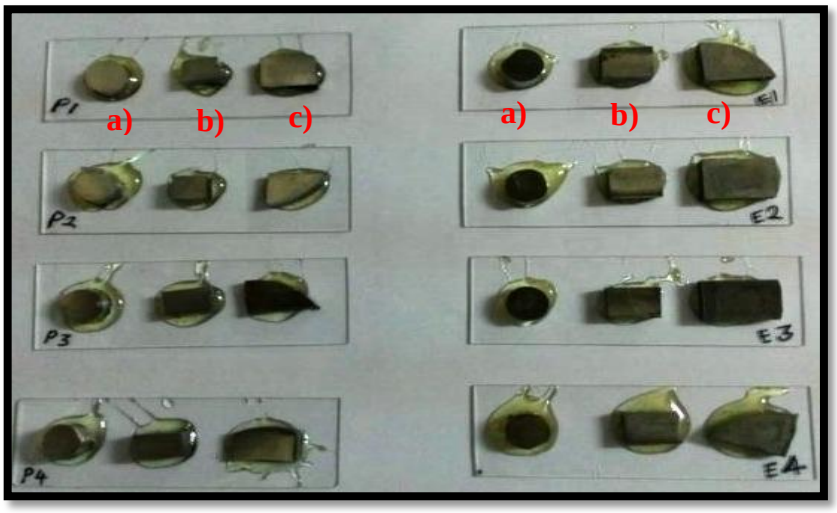

Figure 3-2: Images of the metals used a) Stainless Steel (N690) b) Stainless Steel (AV220SC) c) Titanium

3.4 Methods

3.4.1 Preservation of Stock Culture

Escherichia coli, Bacillus subtilis and *Staphylococcus aureus* were obtained from the Centre Laboratory of University Malaysia Pahang. For long term preservation, the culture was kept in 20% (v/v) glycerol, in a freezer at -80 °C (Zain, 2013). For use in subsequent microbial work, *E. coli, B. subtilis* and *S. aureus* stock were stored in the chiller at 4 - 6 ^{0}C, transferred to an agar plate and incubated for 24 hours at 30 °C.

3.4.2 Media Preparation

3.4.2.1 Preparation of Nutrient Broth

8 g nutrient broth powder made up of 20 g/L glucose, 20 g/L bactopeptone and 10 g/L yeast extract were weighted and adjusted to pH 5.5 by using 0.1 M sulphuric acid and 0.1 M NaOH solution. The powder then added to 1 L of distilled or deionized water in a 1 L Schott bottle. The powder was dissolved in the water and heated for 15 minutes by using hot magnetic plate to make sure it was dissolve completely and finally autoclaved at 121 °C for 20 minutes.

3.4.2.2 Preparation of Nutrient Agar

20 g of nutrient agar powder containing 20 g/L glucose, 23 g/L agar powder, 20 g/L bactopeptone and 10 g/L yeast extract were measured and adjusted to pH 5.5 by using 0.1 M sulphuric acid and 0.1 M NaOH solution. The powder then added to 1 L of distilled and heated for 15 minutes using hot magnetic plate to makes sure it dissolved completely. The solution was autoclaved at 121 °C for 20 minutes and allowed to cool to 50 °C before pouring into the petri dish. The agar was kept in 4 °C freezer until further use.

3.4.2.3 Preparation of Agar Plates

15-20 mL of a warm sterile nutrient agar was poured per petri plate. The nutrient agar then allowed to solidify at room temperature in sterile environment and kept in 4 °C until further use. Figure 3-3 showed agar plates that have been prepared.